Post Treatment Lyme Disease Syndrome and the *SLICE* Study

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Learning Objectives

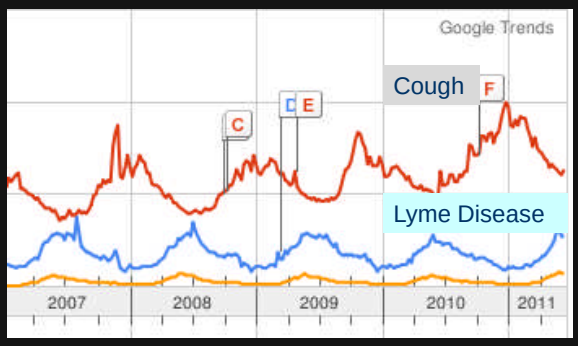
- Review the Natural History and serologic evolution of untreated Lyme disease
- Review the evidence and risk factors for Post Treatment Lyme Disease Syndrome
- Preview the SLICE study, a prospective cohort study now under way at Greenspring Station/Johns Hopkins Bayview

Lyme disease
38,000 confirmed/probable reported cases in 2009

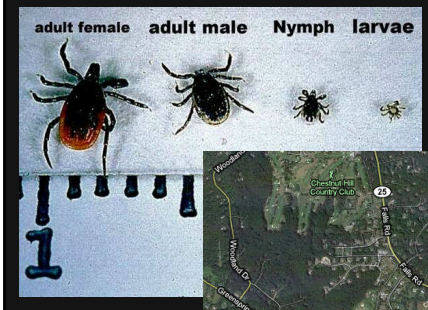
- Seasonal
- Geographic

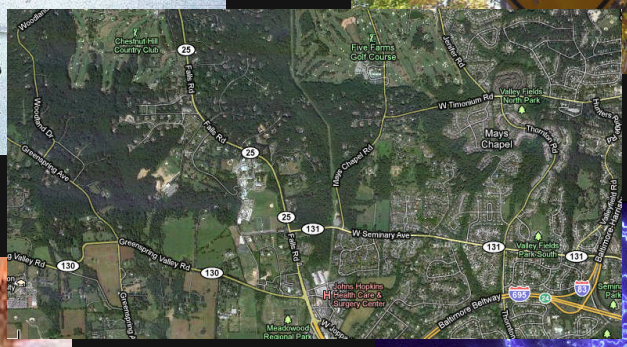
Google Search sites

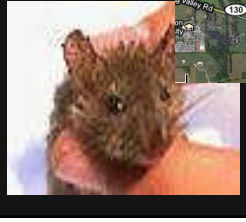
1. Meriden, CT
2. Providence, RI
3. Hartford, CT
4. Albany, NY
5. Boston, MA
6. **Baltimore, MD**

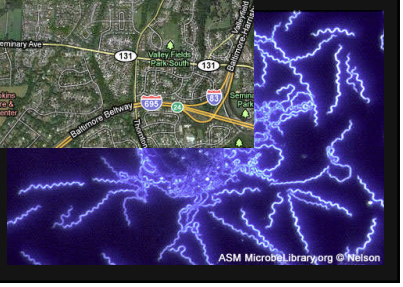


Lyme Country

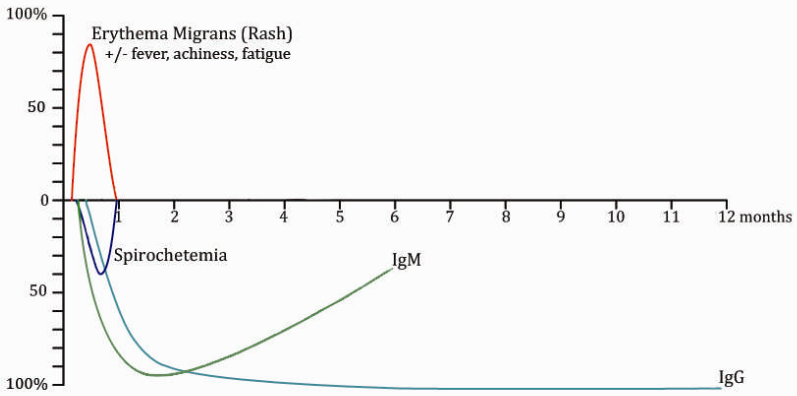




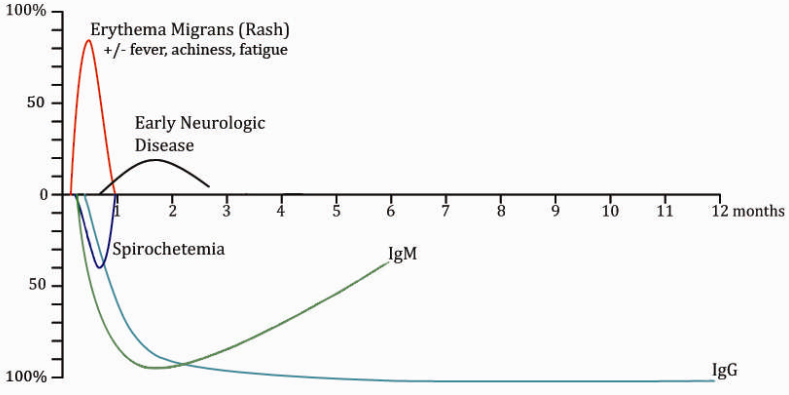




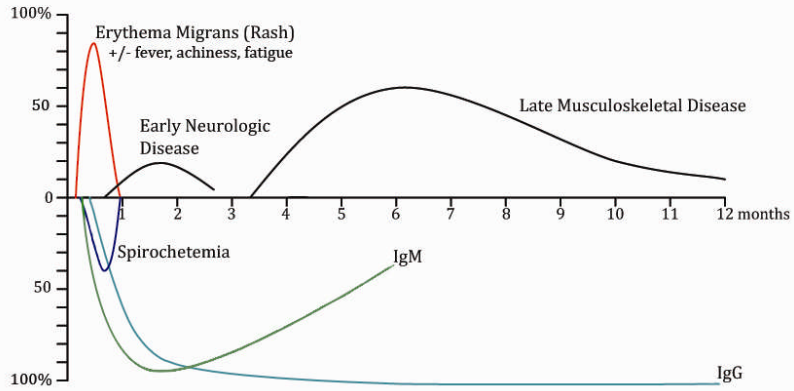
Natural History of Untreated Early Lyme Disease



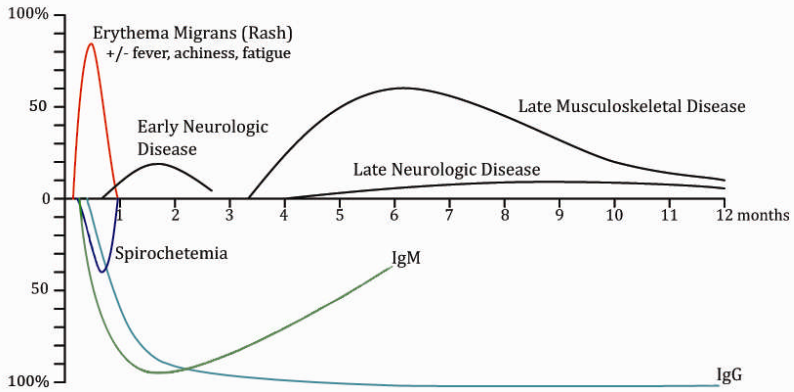
Untreated Lyme Disease – Early Neurologic disease



Untreated Lyme Disease – Late Lyme arthritis



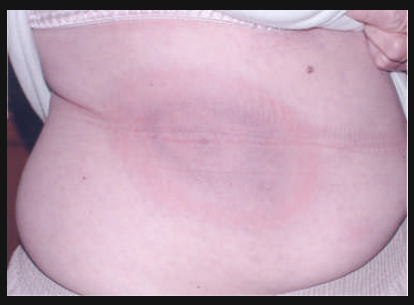
Untreated Lyme Disease – Late Neurologic disease



Diagnosis of Early Lyme Disease is based on the Physician's ability to recognize Erythema Migrans

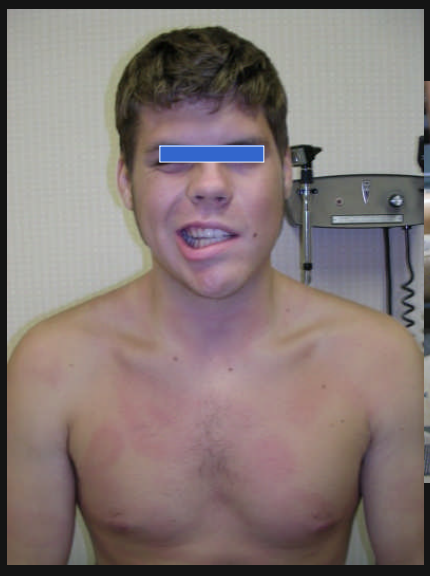
- Physician recognition of diagnostic EM rash is imperfect. Feder H. *Am J Med* ***99:41, 1995.***
  - atypical manifestations of rash possible
  - Lack of recognition results in underdiagnosis
- Disseminated cutaneous skin manifestations not widely recognized
- "Classic bull's eye" is NOT the most common presentation of EM as is commonly believed
  Tibbles C. JAMA 297:2617, 2007

Variable manifestations of EM Rash

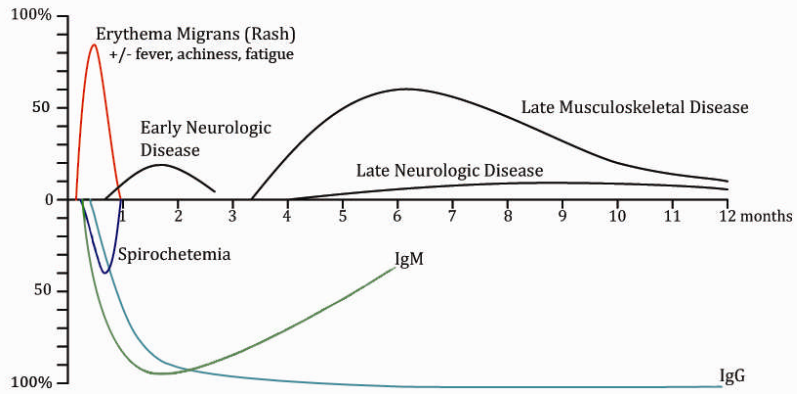



EM may be subtle in appearance

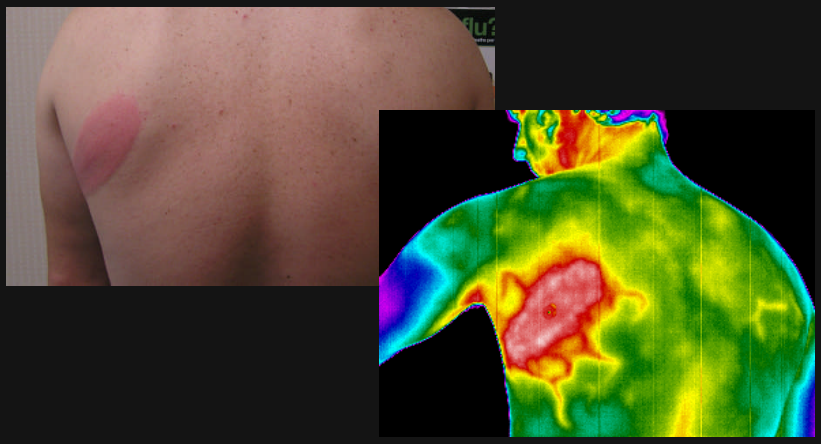
Disseminated Rash of Lyme disease



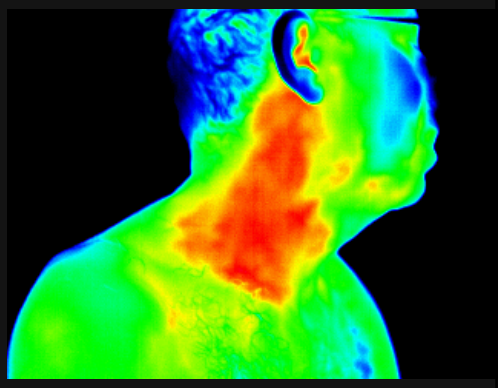
Serology is used to confirm the diagnosis in suspected cases without a diagnostic EM rash



Skin is the initial immune site responding to infection

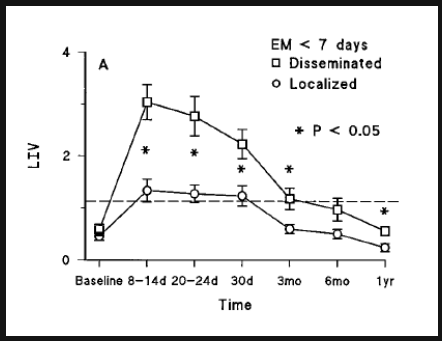


Regional lymph nodes are important for the ongoing immune response



Evolution of the Serologic Response (ELISA) to *Borrelia burgdorferi* in Treated Patients with Culture-Confirmed EM

Antibody response is delayed by several weeks
Higher levels in disseminated disease
Antibody levels fall soon after treatment



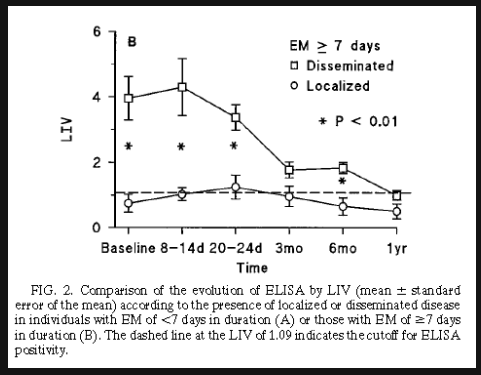
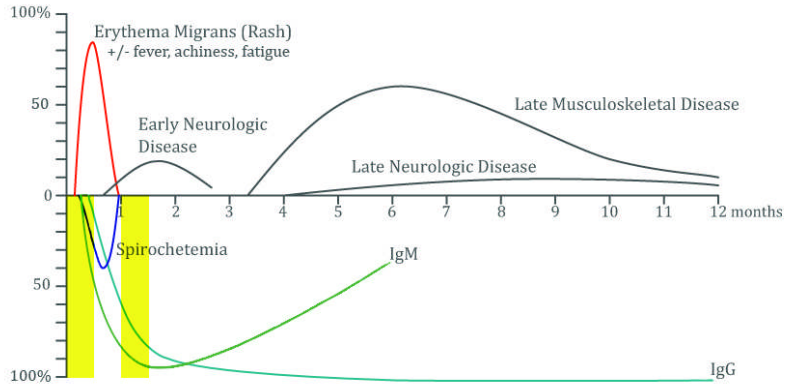


FIG. 2. Comparison of the evolution of ELISA by LIV (mean ± standard error of the mean) according to the presence of localized or disseminated disease in individuals with EM of <7 days in duration (A) or those with EM of ≥7 days in duration (B). The dashed line at the LIV of 1.09 indicates the cutoff for ELISA positivity.

MARIA E. AGUERO-ROSENFELD,et.al

Serologic Windows in IgM and IgG responses



Diagnosis of Lyme Borreliosis

Maria E. Aguero-Rosenfeld,1,4* Guiqing Wang,2 Ira Schwartz,2 and Gary P. Wormser3,4
CLINICAL MICROBIOLOGY REVIEWS, July 2005, p. 484–509

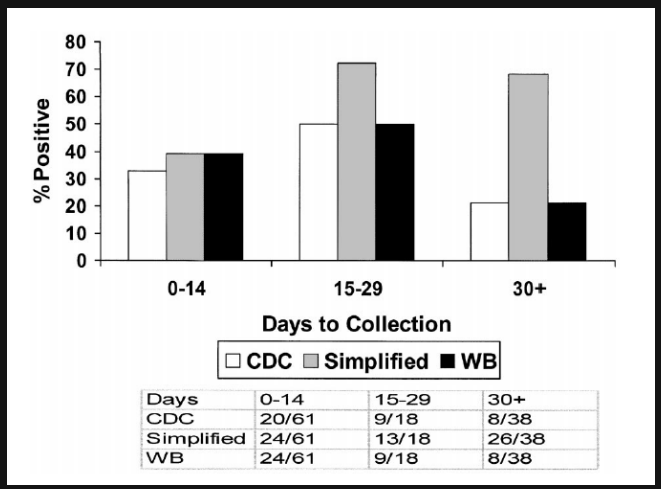
TABLE 4. Reactivities obtained using different methods to detect antibodies to *B. burgdorferi* in Lyme borreliosis in patients from the United States

Test	% Reactivity (reference[s]) in patients with:			
	EM, acute phase	EM, convalescent phase[a]	Neurological involvement	Arthritis
Whole-cell ELISA	33–49 (7, 8, 89, 337)	76–86 (7, 8, 89, 337)	79 (IgG ELISA) (84)	100 (IgG ELISA) (84)
IgM IB[b]	43–44 (7, 89)	75–84 (7, 89)	80[c] (84)	16[c] (84)
IgG IB[b]	0–13 (7), 43.6[d]	15–21 (7), 80[d]	64–72 (84)	96–100 (84)
Two-tier testing	29–40 (7, 15, 89, 227, 337)	29–78 (7, 15, 89, 227, 337)	87 (15)	97 (15)

[a] Sera obtained after antimicrobial treatment.
[b] IgM and IgG IB criteria are those of Engstrom et al. (89) and Dressler et al. (84), respectively, except as indicated.
[c] IgM IB criteria of Dressler et al. (84).
[d] IgG IB criteria of Engstrom et al. (89).

Evaluation of Two-Test Serodiagnostic Method for Early Lyme Disease in Clinical Practice

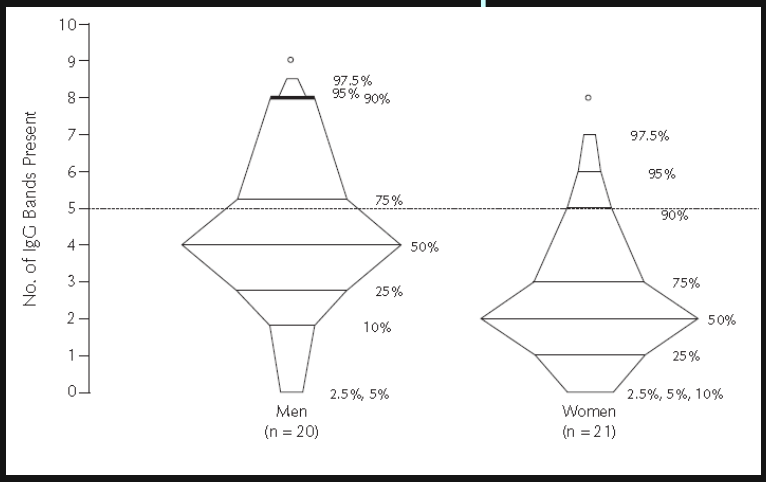
R. T. Trevejo, P. J. Krause, et. al. JID: 1999;179:931–8



Days	0-14	15-29	30+
CDC	20/61	9/18	8/38
Simplified	24/61	13/18	26/38
WB	24/61	9/18	8/38

Figure 3. Sensitivity of CDC-recommended and simplified approaches and Western immunoblotting alone by interval from illness onset to serum specimen collection date (days).

Sex Based Differences in immune response



Sex Differences in the Clinical and Serologic Presentation of Early Lyme Disease: Results From a Retrospective Review.
Alison Schwarzwalder, MPH; Michael F. Schneider, MS; Alison Lydecker, MPH; and John N. Aucott, MD
GENDER MEDICINE/VOL. 7, NO. 4, 2010

Problems with clinical diagnosis and serology may lead to delayed and missed diagnosis and treatment

- *BMC Infectious Diseases*, Jun 1, 2009;9(1):79. Aucott J, Morrison C, Munoz B, Rowe PC, Schwarzwalder A, West SK.

- Retrospective review of 165 patients evaluated for early Lyme disease
- 13% did not present with erythema migrans, but with systemic symptoms alone
  - 54% previously misdiagnosed
- Among those with a rash
  - Dx of EM was missed at initial evaluation in 23% pts
  - 41% had received initial antibiotics likely to be ineffective against Lyme disease

Post Treatment Lyme disease Syndrome

- First Description: Steere et al Ann Int Med 99:22, 1983
  - TCN vs. PCN vs Erythro
  - EM rash resolved in all groups
  - Treatment failure defined by new objective signs
  - However "with all three antibiotic agents **nearly half of the patients had minor late symptoms**"
  - Follow up showed some with symptoms 10-20 yrs later
- Other Observations: Luft et al Ann Int Med 124:785,1996
  - Azithromycin (500mg X 7 days) vs Amoxicillin
  - Post Rx "relapse" with new symptoms in 16% vs. 4%
    - 53% of PTLS patients were seronegative
    - Seronegativity risk factor for relapse

Post Treatment Lyme Disease Syndrome Illness of Subjective Symptoms

- Symptoms of PTLS: Luft et al Ann Int Med 124:785,1996
  - Fatigue 65%
  - Musculoskeletal pain 55%
  - Headache 45%
  - Stiff neck 25%
  - Paresthesias 25%
  - Cognitive dysfunction
- When post treatment symptoms persist for > 6 months called Post Treatment Lyme disease Syndrome

Two Types of Studies Two Different Views of PTLS

– Retrospective Studies of pts previously Rx Lyme

- Lyme Disease Associated with Fibromyalgia. Dinerman, Steere Annals of Internal Medicine. 1992:117:281-285.
- Lyme Disease: An infectious and Postinfectious Syndrome Weinstein A. J Rheum 21:454, 1994
- Musculoskeletal and Neurologic Outcomes Shadick N. Ann Int Med 121:560, 1994 and 131:919, 1999
- NIH Re-Treatment Trial Klempner: NEJM 345:85, 2001

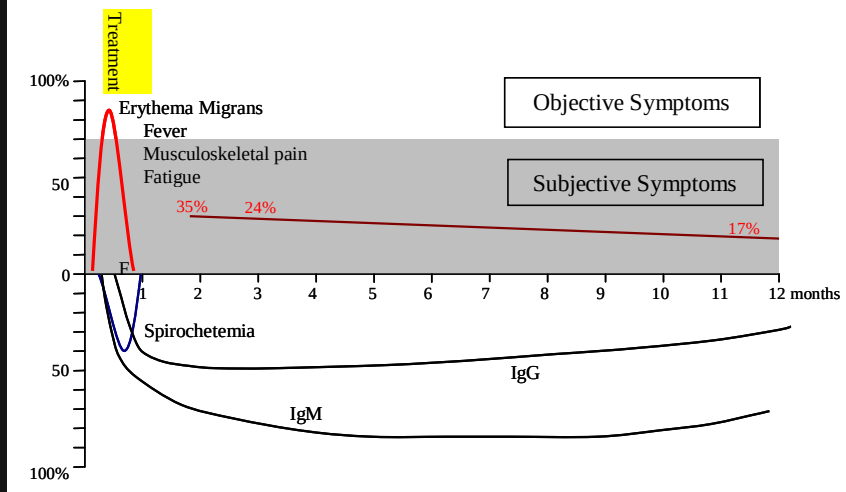
Conclusions of Retrospective Studies

- Retrospective studies, previous Dx Lyme
  - Greater percentage of patients with PTLS (53%)
  - Long-term musculoskeletal and verbal memory impairment and poorer functional status
    - SF-36 measures of health-related quality of life
      - 25% percentile, similar to sciatica, CHF
      - 1–2 SD below matched healthy controls
  - Contradictory Study: Seltzer JAMA 283:609, 2000
    - Same frequency symptoms as control
- Meta Analysis: Cairns V. Int J Epi 34:1340,2005
  - Prevalence of fatigue, pain, cognitive complaints significantly higher (>5%) in pts with Hx of Lyme

Two Types of Studies
Two Different Views of PTLS

- Prospective studies of effective early Antibiotic Treatment of EM pts
  - Decreased rates of PTLS: 4%-16%
  - Patient reported symptoms usually mild
  - Symptoms usually improve over weeks to months
  - 10% patients with symptoms at mean of 5.6 years after treatment
    - Nowakowski,. Wormser, Am J Med. 2003;115:91–96.

Clinical outcomes of treated Early Lyme Disease
Post Treatment Lyme Disease



Problems with Studies

- Prospective studies of EM pts
  - Only enroll patients with most common manifestation of early Lyme (EM), not viral-like illness without rash
  - No controls without Lyme for comparison to PTLS
  - Clinical end points based on physical findings
  - No standardized symptom surveys to quantify subjective symptoms
- Retrospective Studies of pts with treated remote Lyme disease
  - Complex mix of initial illness severity
  - Delayed diagnosis and treatment
  - Patients likely retreated for persistent symptoms
  - Biased for sickest patients

Problems with Studies

- Studies lack ability to analyze impact of known risk factors for PTLS
  - Initial severity of illness
  - Delay in treatment
  - Use of non-ideal antibiotics:
    - Macrolides and TCN
    - non-recommended agents: quinolones, TMP/Sulfa, 1st gen cephalosporins

Confusion and Controversy over PTLS

- Possible explanation:
  - Differences between community and “study care” may limit generalizability of studies
  - Marked differences exist between community care and that in academic medical centers and studies
- No studies in literature of patterns of care in community
  - Assess accuracy of diagnosis and treatment
  - Describe patients with broadest spectrum of illness
- Do risk factors for high rates and severity of PTLS seen in retrospective studies exist today?

Conclusions from our Experience

- Diagnosis and treatment of Lyme disease remains a significant problem
  - Lack of accurate diagnosis of EM
  - Lack of recognition of systemic presentations without rash
  - Difficulty using serology to confirm Dx
  - Non specific antibiotic use is common with high rates of exposure of patients to non-ideal treatment of Lyme disease
- Significant risk factors for PTLS remain in community practice of medicine

PTLS vs. Chronic Lyme

- If risk for PLS in community remains high
  - PTLS may account for some % of diagnoses in patients presenting for evaluation of “Chronic Lyme Disease”
  - If patients seen for Chronic Lyme Disease don’t have PTLS, what do they have?

"Chronic Lyme Disease" in literature refers to a heterogenous group

- Sigal first 100 pts Lyme disease Center:
  - 24 Untreated Lyme
    - 16 Early Lyme disease: EM rash
    - 8 Untreated Late Lyme disease: joint, neurologic
  - 30 Sx probably related to prior treated Lyme disease:
    - 4 recrudescent Lyme
    - 9 with "residual symptoms" post treatment
    - 17 Fibromyalgia after documented Lyme disease
  - 43 Illness with no obvious history of prior Lyme
    - 7 with fibromyalgia unrelated to Lyme
    - 22 other diagnosis: RA (4), Spondylo (4), OA (3)
    - 14 No diagnosis made: Medically unexplained symptoms

How to Diagnose Subset of PTLS in the population of Chronic Lyme Disease

- No reliable marker for prior exposure to Lyme
  - Convalescent serology insensitive for prior disease
  - Significant rate of negative serology with early Rx
- Dx approach: Taking a good history
  - Listening to the patient's symptoms
  - Establishing a history of primary Lyme illness
    - Finding the "spider bite"
  - Establishing treatment history
  - Excluding other diagnosis

Proposed definition of post–Lyme disease syndrome

Inclusion criteria

An adult or child with a documented episode of early or late Lyme disease fulfilling the case definition of the Centers for Disease Control and Prevention [112]. If based on erythema migrans, the diagnosis must be made and documented by an experienced health care practitioner.

After treatment of the episode of Lyme disease with a generally accepted treatment regimen [146] (tables 2 and 3), there is resolution or stabilization of the objective manifestation(s) of Lyme disease.

Onset of any of the following subjective symptoms within 6 months of the diagnosis of Lyme disease and persistence of continuous or relapsing symptoms for at least a 6 month period after completion of antibiotic therapy:

- Fatigue
- Widespread musculoskeletal pain
- Complaints of cognitive difficulties

Subjective symptoms are of such severity that, when present, they result in substantial reduction in previous levels of occupational, educational, social, or personal activities.

Exclusion criteria

An active, untreated, well-documented coinfection, such as babesiosis.

The presence of objective abnormalities on physical examination or on neuropsychologic testing that may explain the patient's complaints. For example, a patient with antibiotic refractory Lyme arthritis would be excluded. A patient with late neuroborreliosis associated with encephalopathy, who has recurrent or refractory objective cognitive dysfunction, would be excluded.

A diagnosis of fibromyalgia or chronic fatigue syndrome before the onset of Lyme disease.

A prolonged history of undiagnosed or unexplained somatic complaints, such as musculoskeletal pains or fatigue, before the onset of Lyme disease.

A diagnosis of an underlying disease or condition that might explain the patient's symptoms (e.g., morbid obesity, with a body mass index [calculated as weight in kilograms divided by the square of height in meters] ≥45; sleep apnea and narcolepsy; side effects of medications; autoimmune diseases; uncontrolled cardiopulmonary or endocrine disorders; malignant conditions within 2 years, except for uncomplicated skin cancer; known current liver disease; any past or current diagnosis of a major depressive disorder with psychotic or melancholic features; bipolar affective disorders; schizophrenia of any subtype; delusional disorders of any subtype; dementias of any subtype; anorexia nervosa or bulimia nervosa; and active drug abuse or alcoholism at present or within 2 years).

Laboratory or imaging abnormalities that might suggest an undiagnosed process distinct from post–Lyme disease syndrome, such as a highly elevated erythrocyte sedimentation rate (>50 mm/h); abnormal thyroid function; a hematologic abnormality; abnormal levels of serum albumin, total protein, globulin, calcium, phosphorus, glucose, urea nitrogen, electrolytes, or creatinine; significant abnormalities on urine analysis; elevated liver enzyme levels; or a test result suggestive of the presence of a collagen vascular disease.

Although testing by either culture or PCR for evidence of *Borrelia burgdorferi* infection is not required, should such testing be done by reliable methods, a positive result would be an exclusion.

Wormser G P et al. Clin Infect Dis. 2006;43:1089-1134

Clinical Infectious Diseases

Conclusions

- Post Treatment Lyme Syndrome Exist
  - Pathophysiology of PTLS is Unclear
  - Risk factor: non-ideal antibiotic treatment, initial severity of illness, delay of diagnosis
- Severity of PTLS: Broad spectrum of illness
  - May be self limited or chronic
- True number of cases of PTLS unknown
  - Serology not useful marker of exposure
  - PTLS may account for subset of pts with "Chronic Lyme"
- Further research needed
  - SLICE study will help in discovery of biomarkers that identify PTLS patients and may lead to Rx options.

SLICE: Study of Lyme Immunology and Clinical Endpoints

Translational Clinical Research Collaboration between Community based practices at
Johns Hopkins at Greenspring Station and
Division of Rheumatology, Johns Hopkins Bayview

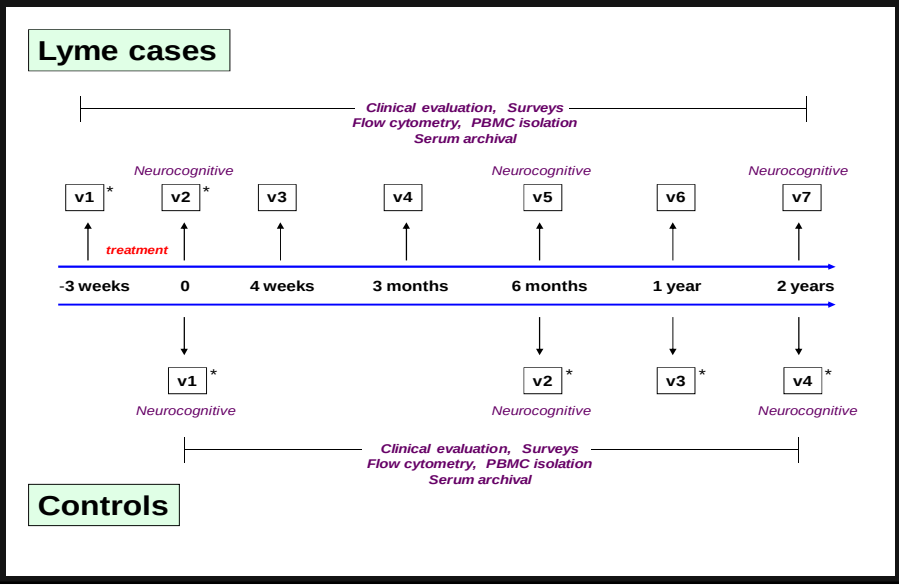
Co-Principal investigators:
Dr. John Aucott and Dr. Mark Soloski
Study Coordinators:
Alison Rebman MPH
Lauren Crowder MPH

Hopkins IRB Application No.: NA_00011170 , Approved May 26, 2009

SLICE Study

- First prospective cohort study with non-Lyme affected controls for comparison
  - 5 year prospective cohort with 2 year follow up
  - At enrollment all patients have documented EM rash
  - Pretreatment and 6 post treatment visits
  - Validated symptom and severity of illness measures
  - Immunophenotyping, biorepository for future studies
- Unique opportunity to study disease process at onset and over time that results in diverse clinical outcomes

The SLICE study is a 2-year prospective cohort study. Data are collected at the following intervals from a cohort of Lyme patients and a cohort of non-Lyme controls:



Greenspring Station

<u>Self-Administered Surveys</u>

- SF-36
- McGill Pain Questionnaire
- Fatigue Severity Scale
- Pittsburgh Sleep Quality Index
- Beck Depression Inventory
- Life Events subscale

<u>Neurocognitive</u>

- Word Reading Subtest (WRAT4)
- Digit Span
- STROOP Color-Word
- Symbol Digit
- Hopkins Verbal Learning Test
- Trail Making A & B
- Controlled Word (COWAT)

Bayview Medical Center

- Flow Cytometry to measure cellular immune response
- Biorepository:
  - Serum
  - DNA/RNA
  - PBMC isolation
- Microbial PCR

Current Enrollment – 2/1/11

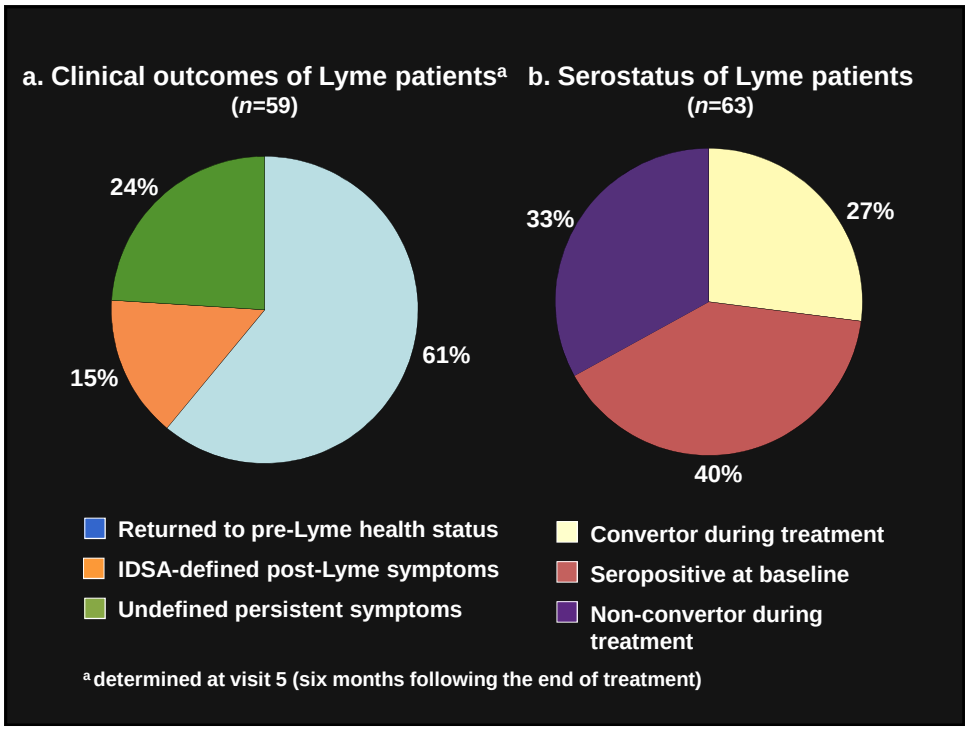
65 acute Lyme cases
- 1 documented co-infection
- 6 retreated after completing initial therapy
  - 3 IV for neuropathy by (+) nerve conduction studies
  - 3 enlarging primary rash on initial therapy

3 late Lyme cases
14 enrolled controls

416 participant-visits completed (specimens obtained)

81 visit 1	67 visit 4	14 visit 7
78 visit 2	63 visit 5	
70 visit 3	43 visit 6	

99% retention rate following treatment (visit 2)
97% retention rate after 6 months (visit 5)



SF-36

Standardized measure of Quality of Life made up of 8 subscales

	IDSA PLS (-)[a] n=30	IDSA PLS (+)[a] n=7	*p*
Physical Functioning	57.0	52.8	0.02[b]
Role Physical	56.9	47.1	<0.01[b]
Body Pain	55.4	46.1	0.21
General Health	52.9	45.8	0.44
Vitality	58.3	39.6	<0.01[b]
Social Functioning	56.9	51.4	0.04
Role Emotional	55.9	36.4	<0.01[b]
Mental Health	55.6	38.7	0.27

[a] median reported
[b] also significantly different ($p<0.05$) from median control score (n=11)

"Immunocellomics" of Lyme

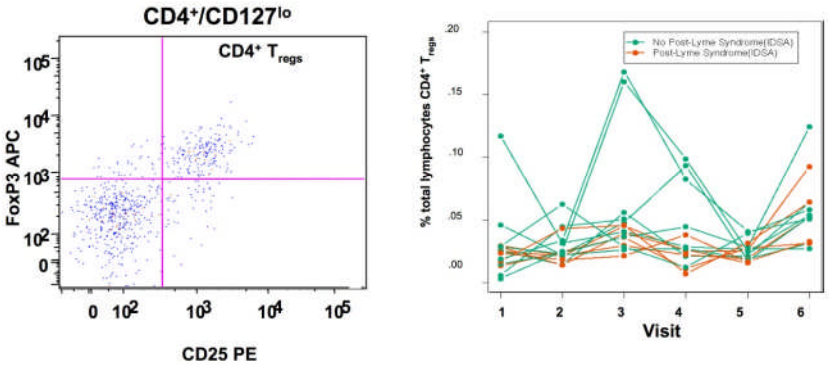
**Mark Soloski,PhD, Director flow Cytometry Lab,
Johns Hopkins Bayview Campus**

Table 1: Monoclonal Antibody Panels to Detect Immune Cell Subsets.

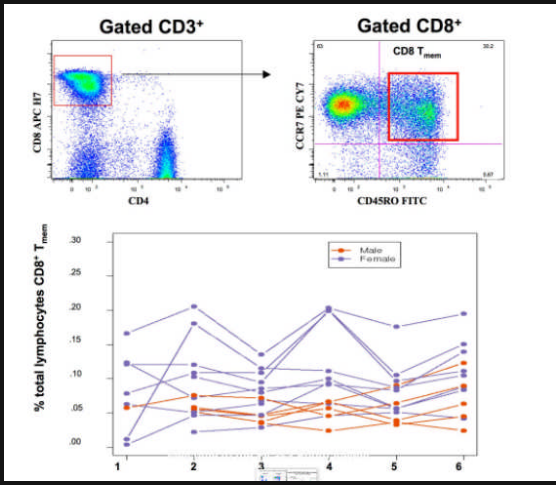
Panel A T Cell Subsets	Panel B Innate Immune Cells	Panel C T Regulatory Cells	Panel D T Cell Skin Homing	Panel E T Cell Activation	Panel F B cell	Panel G Dendritic Cells
CD3	TCR$\alpha\beta$	CD3	CD3	CD3	CD19	Lineage mix*
CD4	TCR$\gamma\delta$	CD4	CD4	CD4	CD5	CD11c
CD8	CD8	CD25	CD8	CD8	IgM	HLA-DR
CCR7	CD56	CD127	CXCR3	CD69	IgD	CD40
CD45RO	CD57	FoxP3	CCR3	CD25	CD20	CD80
CD27	CD16		CCR4	HLA-DR	CD23	CD86
CD28	CD107a		CCR10	CD137	CD24	CD83
CCR5			CCR5	CD154		CD123
CRTH2				CD107a		CD14
CCR4						
CCR6						

* (cocktail of anti-CD3, CD19, CD20, CD16)

Levels of CD4⁺ CD25⁺/FoxP3⁺ cells are increased only in non-PLS Patients



CD8 Tmem PBMCs increased in women vs. men



Future Trends in Lyme Disease